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Ion-Pair Chromatography

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Introduction

In ion pair chromatography, the solute ion is distributed between the mobile and the stationary phase together with an ion of opposite charge (a so-called counterion). The technique is often used in reversed-phase chromatography as a convenient method to control the retention of solutes. The principle may, for example, be applied to direct chiral separations using either chiral or achiral counterions. When using an achiral counterion, the chromatographic system has to contain a chiral selector, e.g., a chiral stationary phase. The purpose of an achiral counterion then is to control the retention of the solute. However, the counterion may also influence the stereoselectivity by interactions with the chiral selector molecules. A chiral counterion may, on the other hand, be used with non-chiral stationary phases to promote chiral separation.

Ion Pairs: Principles

In order to distribute the solute molecules in a nonpolar environment, it has to be uncharged. However, if an ion of opposite charge is present in enough concentration the two ions may be distributed as a pair in the same way. An ion being double charged may be distributed together with two ions of single charge or one double charged, the only prerequisite being electroneutrality of the pair (**Figure 1**).

The equilibrium for a simple 1 : 1 ion pair may be given as:

$$HB^+ + C^- \leftrightarrow HBC$$

and is characterized by an extraction constant:

$$K_{ex} = \frac{[HBC]_{org}}{[HB^+]_{aq} \times [C^-]_{aq}} \quad [1]$$

The nonpolar environment may be a liquid (mobile or stationary), a surface or a micelle in the liquid. For simplicity the principle for a liquid–liquid chromatographic system with aqueous mobile phase will be presented. Here the retention factor for the solute, k_{HB^+}, is equal to:

$$k_{HB^+} = D_{HB^+} \times (V_s/V_m) \quad [2]$$

where D_{HB^+} is the distribution coefficient of the solute between the aqueous mobile phase and the organic stationary phase and V_s and V_m are the volumes of the two phases.

The distribution coefficient is defined as:

$$D = \frac{C_{org}}{C_{aq}} \quad [3]$$

i.e., the ratio of the total concentration of solute in organic phase over the total concentration in aqueous phase. For the ion pair, D_{HB^+} may be expressed as:

$$D_{HB^+} = \frac{[HBC]_{org}}{[HB^+]_{aq}} = K_{ex} \times [C^-]_{aq} \quad [4]$$

Thus, the retention factor will depend on the extraction constant of the ion pair and the concentration of counterion in the aqueous mobile phase. The magnitude of the extraction constants depends on the hydrophobicity of the solute and the counterion, the

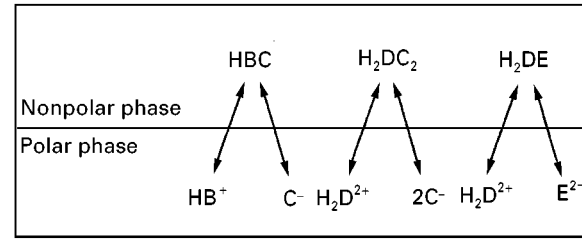

Figure 1 Distribution of charged compounds to a nonpolar phase together with a counterion.

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kind of interaction forces between the two ions, and the physiochemical properties of the organic phase.

For protolytic solutes, the retention will also depend on the pH of the aqueous phase, as the charge of the solute is pH dependent. At a pH where an amine is uncharged, its distribution coefficient is:

$$D_B = \frac{[B]_{org}}{[B]_{aq}} = K_{D(B)} \quad [5]$$

where $K_{D(B)}$ is the distribution constant of B.

Considering a more general expression for the distribution coefficient for a basic compound over the entire pH range in the presence of a counterion, the equation is as follows:

$$D = \frac{C_{org}}{C_{aq}} = \frac{[B]_{org} + [HBC]_{org}}{[B]_{aq} + [HB^+]_{aq}} = \underbrace{\frac{K_{D(B)}K'_{(HB^+)}}{K'_{(HB^+)} + a_{H^+}}}_{\text{Uncharged form}} + \underbrace{\frac{K_{ex(HBX)}[C^-]_{aq}a_{H^+}}{K'_{(HB^+)} + a_{H^+}}}_{\text{Ion pair}} \quad [6]$$

where $K'_{(HB^+)}$ is the acid dissociation constant of HB^+ defined as:

$$K'_{[HB^+]} = \frac{[B] \cdot a_{H^+}}{[HB^+]} \quad [7]$$

Equation [6] means that the retention of a solute may be made up of two parts: the retention as an ion pair and the retention in an uncharged form:

$$k = k_{uncharged} + k_{ion\ pair} \quad [8]$$

In chromatographic systems with solid stationary phases, the retention involves distribution with a solid surface. The limited capacity of the surface and the possibility to have competition for the limited adsorption sites have to be considered in the equation but the models otherwise resemble each other to a large extent.

Principles of Chiral Separation Using Achiral Counterions

The achiral counterion itself does not provide stereochemical interactions and thus it is expected to only influence the retention and not the separation of an enantiomeric pair. Different counterions have been added to the aqueous mobile phase in a liquid–liquid chromatographic system with a nonpolar chiral stationary phase (a tartaric acid ester). The enantioselectivity was about the same for all counterions studied and, as expected, the type of ion and its concentration could be used to control the retention (**Table 1**).

Several of the chiral selectors used are, however, complex biomolecules, e.g., proteins which cannot be treated as simple organic phases. The addition of charged (and uncharged) compounds to the mobile phase, when a protein is used as the stationary phase, may change both the retention and the enantioselectivity. The changes in enantioselectivity may be dramatic; the two enantiomers in a pair may even elute in an opposite order.

Principles of Chiral Separation Using Chiral Counterions

The chiral counterion may be the only chiral compound needed for separation of enantiomers in a chromatographic system. The solute enantiomers form diastereomeric ion pairs with the chiral counterion, also called the selector (**Figure 2**). In order to achieve enantioselectivity, the ion pairs formed should have different stabilities and/or different distribution properties with the stationary phase. If the difference in distribution properties with the stationary phase is responsible for the stereoselectivity, it is important that the retention as a diastereomeric ion pair (k_{chiral}) is more pronounced than the retention of

Table 1 Influence of counterion structure on retention and enantioselectivity

Solute	–		*ClO_4^- (45 mM)*		*PF_6^- (45 mM)*		*Heptane SO_3^- (45 mM)*		*Hexane SO_3^- (45 mM)*		*Hexane SO_3^- (90 mM)*	
	α	*k*	α	*k*	α	*k*	α	*k*	α	*k*	α	*k*
N-methylephedrine	1.14	1.1	1.13	2.2	1.13	4.6	1.11	7.3	1.12	3.4	1.10	6.2
Ephedrine	1.15	1.1	1.15	2.2	1.15	4.0	1.13	7.7	1.41	3.4	1.13	6.9
Norephedrine	1.16	1.2	1.16	2.5	1.16	4.2	1.14	9.6	1.15	4.1	1.14	8.6

Solid phase: Novapak Phenyl (4 μm-particles); liquid stationary phase, (2*R*,3*R*)-di-n-butyltartrate; mobile phase, counterion in phosphate buffer pH 2.8 (ionic strength 0.1). PF_6^- = hexafluorophosphate, Heptane SO_3^- = heptanesulfonate, Hexane SO_3^- = hexanesulfonate.

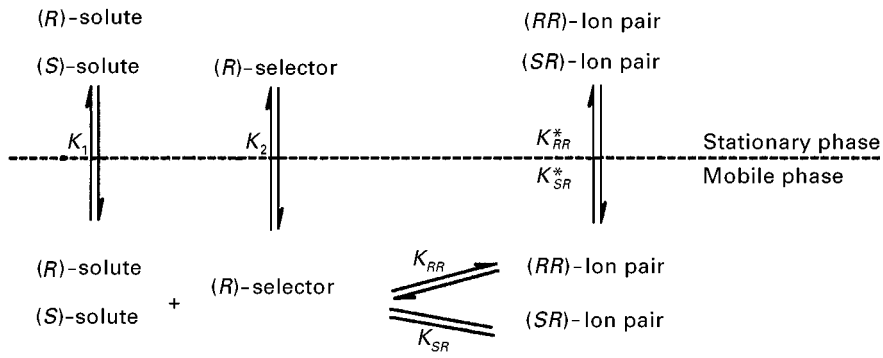


Figure 2 Ion pair formation in mobile phase and possible distribution to stationary phase.

the solute alone (k_{achiral}) (**Figures 2** and **3**). Referring to eqn [8] the retention factor may be described as:

$$k = k_{\text{achiral}} + k_{\text{chiral}} \qquad [9]$$

and the observed α value ($\alpha_{R/S}$) compared to the maximal α (only chiral retention) as:

$$\alpha_{R/S} = \frac{k_R}{k_S} = \frac{\alpha_{\max} + (k_{\text{achiral}}/k_{S(\text{chiral})})}{1 + (k_{\text{achiral}}/k_{S(\text{chiral})})} \qquad [10]$$

where the *S*-form is eluted first. Therefore, the concentration of selector has to reach a certain level to assure maximal stereoselectivities (α values).

In order to promote the formation of an ion pair in the mobile phase, solvents of low polarity have often been used, e.g., hexane and dichloromethane. The retention and stereoselectivity is controlled by the temperature, the mobile phase composition (i.e. type and concentration of counterion, competing substances and polar modifiers) as well as the choice of stationary phase.

The chiral counterion and its concentration influences the equilibrias outlined in **Figure 2**. Equation [11] expresses the stereoselectivity when varying the counterion concentration. From eqn [11] it is obvious that the influence of selector concentration on enantioselectivity is difficult to predict. The effect of a changed selector concentration will depend on the relative magnitudes of ion pair formation constants in the mobile phase and stereoselective distribution to the stationary phase:

$$\alpha_{S/R} = \frac{(K_1 + K_1K_{RR}[(R)\text{–Selector}]_{\text{mob}} + K_{SR}K^*_{SR}[(R)\text{–Selector}]_{\text{mob}} + K_{SR}K_{RR}K^*_{SR}[(R)\text{–Selector}]^2_{\text{mob}})}{(K_1 + K_1K_{SR}[(R)\text{–Selector}]_{\text{mob}} + K_{RR}K^*_{RR}[(R)\text{–Selector}]_{\text{mob}} + K_{SR}K_{RR}K^*_{RR}[(R)\text{–Selector}]^2_{\text{mob}})} \qquad [11]$$

K_1 = adsorption constant for achiral adsorption of solute.
K_{RR} = formation constant of (*R*)–Solute : (*R*)–Selector in mobile phase.
K_{SR} = formation constant of (*S*)–Solute : (*R*)–Selector in mobile phase.
K^*_{RR} = adsorption constant of the diastereomeric ion pair (*R*)–Solute : (*R*)–Selector.
K^*_{SR} = adsorption constant of the diastereomeric ion pair (*S*)–Solute : (*R*)–Selector.
$[(R)\text{–Selector}]_{\text{mob}}$ = concentration of chiral selector in the mobile phase.

The chiral selector does not have to be optically pure, as the interactions are noncovalent. An enantiomerically impure selector will affect the stereoselectivity but only an impurity of exactly 50% may ruin it completely. The expected stereoselectivity, $\alpha^*_{S/R}$ when using enantiomerically impure selectors in the mobile phase may be calculated from the following expression:

$$\alpha^*_{S/R} = \frac{\alpha_{S/R}[(R)\text{–Selector}]_{\text{mob}} + [(S)\text{–Selector}]_{\text{mob}}]}{[(R)\text{–Selector}]_{\text{mob}} + \alpha_{S/R}[(S)\text{–Selector}]_{\text{mob}}]} = \frac{\alpha_{S/R}P + [100 - P]_{\text{mob}}}{P + \alpha_{S/R}[100 - P]_{\text{mob}}} \qquad [12]$$

where $\alpha_{S/R}$ is the stereoselectivity obtained when the selector is present in one pure enantiomeric form (here the *R*-form) and *P* is the fraction of selector present in the *R*-form. Experiments have shown good agreement with theory (**Table 2**).

Chiral Counterions Used

In order to obtain chiral discrimination of the solutes, the counterion and solute ions have to establish multipoint interactions where the stability of the interactions differ between the enantiomers. For the stability of the diastereomeric ion pair, it is advantageous if the molecules involved have a rigid structure, and most of the chiral counterions used are rigid. At least three points of interaction for one of the

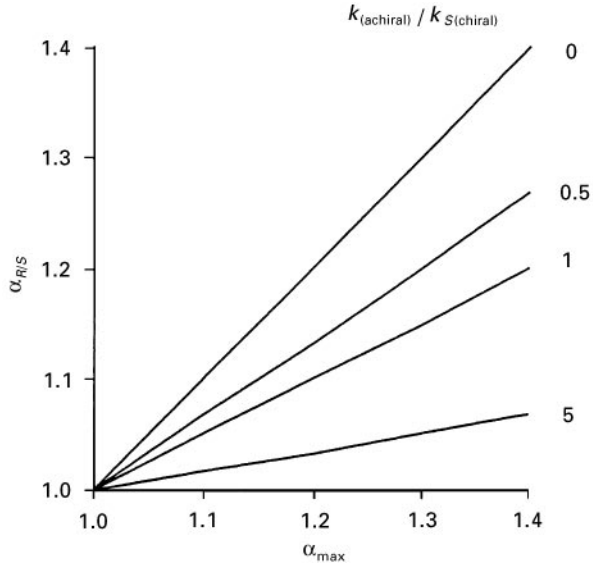


Figure 3 Influence of achiral retention on the separation factor for the enantiomers. (Reprinted from Heldin E. (1991) Tartaric acid derivatives as chiral selectors in liquid chromatography. *Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy* 74: 1–37, with permission from Acta Universitas Upsaliensis.)

enantiomers are necessary for recognition of the three-dimensional structure. All these points do not, however, have to be only between selector and solute but may also involve solvent molecules and the achiral packing material.

Most of the early studies with chiral ion-pairing selectors in the mobile phase were performed with a silica-based DIOL stationary phase. The DIOL functionality was chosen after comparing several phases (nitrile, nitro, C2, silica) as it gave the most symmetrical peaks. Uncoated silica has in some cases been used for the purpose of retaining hydrophilic compounds. As the solid phase, i.e., the particle surface, plays an important role in the chiral recognition process it should be considered in the optimization process. The introduction of porous graphitic carbon (PGC) as packing material thus opened new possibilities and has broadened the use of chiral mobile phase additives. PGC is a very hydrophobic material whose unique three-dimensional structure is suitable for the recognition of isomers. Another advantage is that it may be used with more polar mobile phases, e.g., methanol–water mixtures. The use of polar mobile phases has several advantages in the separation process, among them the possibility of direct injection of biological fluids.

Table 2 Influence of enantiomeric purity of counterion on stereoselectivity

D-form of counterion (%)	$\alpha^*_{S/R}$	
	Calculated	*Found*
0	–	1.38
5	1.34	1.33
10	1.29	1.28
15	1.23	1.26
50	1.00	1.01
100	0.72[a]	0.72[a]

[a] $\alpha^*_{R/S} = 1.38$. Solid phase: LiChrosorb DIOL. Mobile phase: 2.5 mM mixtures of benzoxycarbonylglycyl-L- and D-proline and 0.25 mM triethylamine in dichloromethane (80 ppm water). $\alpha^*_{S/R} = k'$ for (*S*)-propranolol/k' for (*R*)-propranolol. Results reproduced from Pettersson C, Karlsson A and Gioeli C (1987). Influence of enantiomeric purity of a chiral selector on stereoselectivity. *Journal of Chromatography* 407: 217–229, with permission from Elsevier Science.

(+)-10-Camphorsulfonic Acid and Analogues

(+)-10-Camphorsulfonic acid [I] has been used to separate the enantiomers of amino alcohols, mainly β-blocking agents (derivatives of 1-aryl-2-amino alcohols). These solutes have an aromatic part to which a side chain is connected. The selectivity has been found to be dependent on the distance between the hydroxyl and the amine groups, implying that both these structural features play an important role in chiral recognition. (+)-10-Camphorsulfonic acid is an aprotic acid and it also contains an oxo group which may take part in hydrogen bonding. A model for the interaction between (+)-10-camphorsulfonic acid and β-blocking agents has been suggested and is shown in **Figure 4**. Several chiral separations have been performed using (+)-10-camphorsulfonic acid in dichloromethane : 1-pentanol (199 : 1) as mobile phase and a DIOL stationary phase. When using a DIOL functionalized packing material and sulfonic acid in the mobile phase, it is of vital importance to choose a solvent with a low content of polar components as the mobile phase in order to promote ion pair

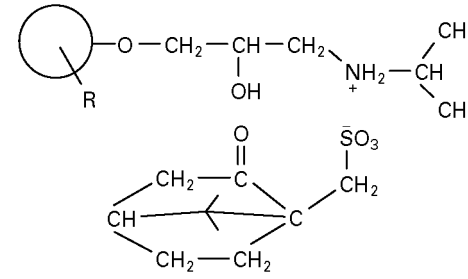
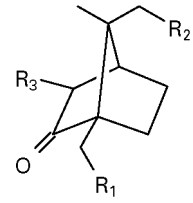


Figure 4 Model for interaction between the (+)-10-camphorsulfonic acid and amino alcohols. (Reprinted from Pettersson C and Schill G (1986) Separation of enantiomers in ion-pair chromatographic systems. *Journal of Liquid Chromatography* 9: 269–290, by courtesy of Marcel Dekker, Inc.)

formation. Attempts to use Br-3-(+)-10-camphorsulfonic acid in a system with a DIOL stationary phase, in order to separate β-blocking agents, have not been successful. With these solutes and in that particular system, the Br group was believed to sterically prevent the three-point contact between the selector and the solute. Moving the sulfonate group away from the oxy group to position 8 (Br-3-(+)-8-camphorsulphonic acid) restored the stereoselectivity to some extent.

[I]



	R_1	R_2	R_3
(+)-10-Camphorsulfonic acid	SO_3H	H	H
(+)-3-Br-10-Camphorsulfonic acid	SO_3H	H	Br
(+)-3-Br-8-Camphorsulfonic acid	H	SO_3H	Br

The selector has been used in a system with porous graphitic carbon (PGC) as support in order to separate some dihydropyridines. The enantiomers of amlodipine and an analogue, UK52.829, were separated with a mobile phase consisting of 5×10^{-3} M (+)-10-camphorsulfonic acid in dichloromethane : methanol (25 : 75) (**Figure 5**). As stated above, PGC may be used together with polar mobile phases. A fairly high content of dichloromethane is needed in order to elute the dihydropyridines from the highly retentive surface of PGC. Exchanging (+)-10-camphorsulfonic acid for Br-3-(+)-10-camphorsulfonic acid still separated the enantiomers although with somewhat lower selectivities.

Cinchona Alkaloids

The cinchona alkaloids are rigid molecules containing four chiral carbons [**II**] and their use as chiral selectors have been studied. Quinine has the absolute configuration 3(*R*),4(*S*),8(*S*),9(*R*) while quinidine has 3(*R*),4(*S*),8(*R*),9(*S*). It is at the positions 8 and 9 that the chiral recognition is believed to take place due to hydrogen bonding groups, and the exchange of quinine for quinidine reverses the retention order of some enantiomers, e.g., 10-camphorsulfonic acid and O-methylmandelic acid (**Table 3**). The mechanism of chiral recognition is, however, not simple as the effect of an exchange of the hydroxyl for an ethylcarbonate group at position 9 reduces the enantioselectivities but not to the same extent for all solutes. Furthermore, the exchange of an ethoxy group for hydrogen at the aromatic moiety (cinchonidine) also had an influence on the stereoselectivity. The results in

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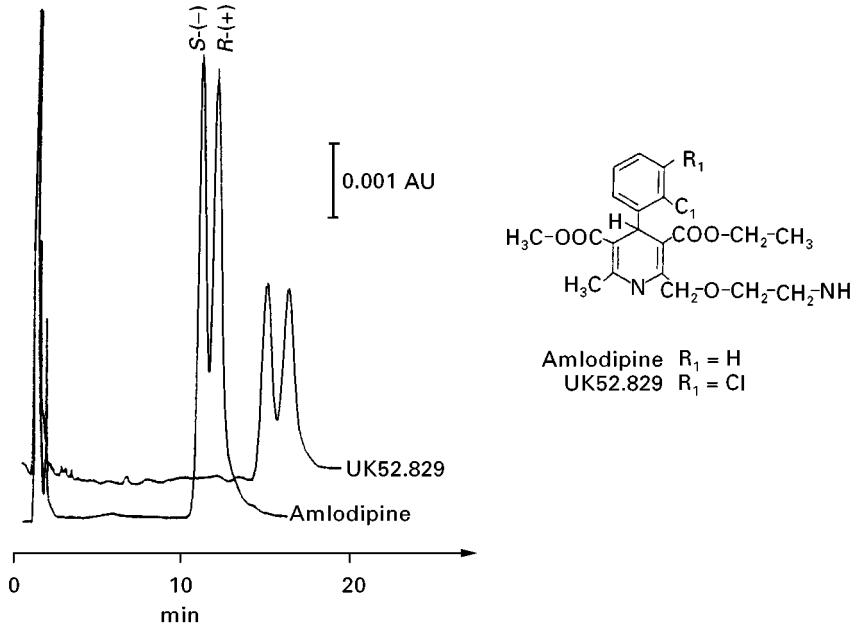


Figure 5 Separation of racemic amlodipine and UK52.829 on Hypercarb-S™. Column temperature: 30°C. Mobile phase: 5 mM (1*S*)-(+)-10-camphorsulfonic acid in dichloromethane–ethanol (25 : 75, v/v). Flow-rate: 1.5 mL min^{-1}. (Reprinted from Josefsson M, Carlsson B and Norlander B (1994) Chiral ion-pair chromatographic separation of two dihydropyridines with camphorsulfonic acids on porous graphitic carbon. *Journal of Chromatography* 684: 23–27, with permission from Elsevier Science.)

Table 3 Influence of counterion structure on retention and enantioselectivity

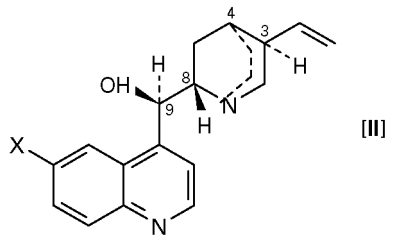
Solutes	*Quinine ethyl carbonate*		*Quinine*		*Quninidine*		*Cinchonidine*	
	α	k_1	α	k_1	α	k_1	α	k_1
10-Camphorsulfonic acid	1.28	1.7(+)	1.47	5.2(−)	1.30	3.0(+)	1.24	6.7(−)
N-(1-Phenylethyl)phthalamic acid	1.01	12.9[a]	1.14	6.7(+)	1.15	4.2(−)	1.18	9.6(+)
O-methylmandelic acid			1.12	7.8(−)	1.18	3.8(+)	1.02	8.7[b]
α-Methoxy-α-trifluoromethylphenylacetic acid	1.09	1.7[a]	1.16	2.4(−)	1.10	1.1(+)	1.0	2.6

Solid phase: LiChrosorb DIOL. Mobile phase: 0.35 mM chiral amine and 0.35 mM acetic acid in dichloromethane (dry) : 1-pentanol (99 : 1). $\alpha = k'_2/k'_1$.

[a]Mobile phase consisted of 0.35 mM quinine ethyl carbonate and 0.35 mM acetic acid in dichloromethane (dry) : n-hexane : 1-pentanol (49 : 50 : 1). Results from Pettersson C and No K (1983) *Journal of Chromatography* 282: 671–684 and Pettersson C (1984) *Journal of Chromatography* 316: 553–567 with permission from Elsevier Science.

[b]Retention order uncertain.

Table 3 come from two different mobile phases. Stereoselectivities should be comparable, as n-hexane does not have a significant effect on enatioselectivity. A high content of n-hexane is needed to retain hydrophilic compounds.



[II]

Quinine	X=OCH_3	3(*R*),4(*S*),8(*S*),9(*R*)
Quinidine	X=OCH_3	3(*R*),4(*S*),8(*R*),9(*S*)
Cinchonidine	X=H	3(*R*),4(*S*),8(*S*),9(*R*)

The complexity of the systems is high as the solutes may form ion pairs in the mobile phase, as well as be distributed to the stationary phase both as ion pairs and as uncharged compounds (see Figure 2). In addition, the selectors may be distributed to the stationary phase alone or together with mobile phase ions. In the cinchona systems, the mobile phase often contains an acid to control the retention and the stereoselectivity of the acidic solutes. It is not possible to provide a simple guideline to know which acid to use as the effect on enantioselectivity differs between the solutes studied. The distribution to the packing material is important and influences both the retention and the enantioselectivity.

When optimizing chiral ion pair separations it is therefore important to carefully consider both the mobile phase and the packing material. Enantiomers of *N*-blocked amino acids, sulfonic acids and carboxylic acids have been separated with the cinchona alkaloids as mobile phase additives. An example of the resolution of D- and L-dansyl-valine is shown in **Figure 6**.

Peptides

The first peptide used for a chiral separation was L-leucine-L-leucine-L-leucine, a zwitterionic compound. Some amino acids, including, D,L-tryptophan and glycyl-D,L-phenylalanine, have been partially resolved by reversed-phase chromatography. The *N*-protected dipeptide *N*-benzyloxycarbonyl-glycyl-L-proline (L-ZGP), shown in **Table 4**, has been used successfully as a counterion in the separation of enantiomers of amines. When bare silica or modified silicas are used as packing material, an enantiomeric pair generally has to be a β-amino alcohol in order to be separable. In order to separate the enantiomers of amines (which lack a strong hydrogen-bonding group in the vicinity of the asymmetric carbon atom) or rather hydrophilic amino alcohols, PGC is a good choice of stationary phase. Most recent separations have been performed on PGC material and a large number of different enantiomers have been shown to be separable, of which some examples are given in **Table 5**. The influence of the structure of the chiral selector on the enantioselectivity of alprenolol has been studied using both DIOL functionalized particles and PGC as packing material. In the DIOL system, one analogue of ZGP with a esterified acid function (L-ZGP methyl ester) and one lacking the glycyl group (Z-L-proline) have been tested. It was found that the acidic function is needed and that this function, together with the keto group in the benzyloxycarbonyl group, was enough for enantioselectivity. Exchanging the glycyl in L-ZGP for L-alanyl will, however, completely ruin the stereoselectivity and the methyl group in alanyl is believed to block the hydrogen-accepting

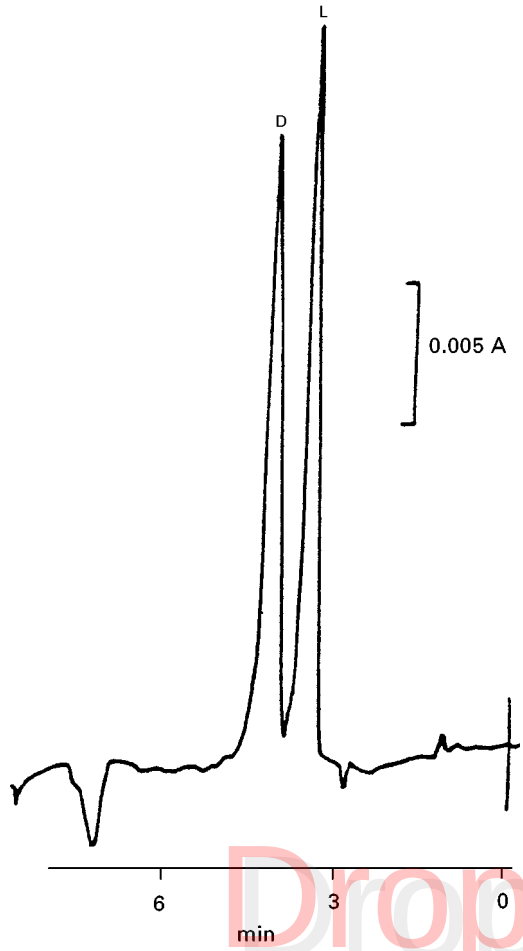


Figure 6 Resolution of D,L-dansyl-valine. Solid phase: LiChrosorb DIOL. Mobile phase: Quinine and acetic acid 0.35 mM in dichloromethane (dry) + 1-pentanol (99 + 1). (Reprinted from Pettersson C (1984) Chromatographic separation of enantiomers of acids with quinine as chiral counterion. *Journal of Chromatography* 316: 553–567, with permission from Elsevier Science.)

keto group. Reversing the positions of alanine and proline restores the selectivity to some extent (**Table 4**).

The aromatic function in L-ZGP provided by the *N*-blocking benzyloxycarbonyl group seems not to be a prerequisite for chiral recognition as a low enantioselectivity has been observed for some solutes using *N*-cyclopentylpropionyl-L-proline as chiral selector. Thus, when using a DIOL stationary phase, chiral recognition is believed to involve an electrostatic attraction to the acidic function in L-proline and hydrogen bonding to the carbonyl group.

Several selectors structurally related to L-ZGP have been studied in systems with PGC as support. In **Table 6**, the results from one of these studies is presented. L-ZGP, *N*-benzyloxycarbonyl-glycyl-glycyl-L-proline (L-ZGGP) and 1-[(2S)-3-mercapto-2-methylpropionyl)-L-proline (captopril) may be regarded as three supplementary selectors. Although all three show enantioselectivity for metoprolol, L-ZGP is not 'usable' for metoprolol analogues having a distance larger than two carbons between the hydroxyl and amine function. In order to match the increase in the distance between possible points of interaction, introduction of a hydrogen-bonding group further apart in the selector promotes the enantioselectivity.

Recently, two new *N*-derivatized dipeptides, *N*-benzyloxycarbonyl-L-glutamyl-L-proline (Z-L-Glu-L-Pro) and *N*-benzyloxycarbonyl-L-glutamyl-D-proline (Z-L-Glu-D-Pro), have been reported. Only one of the dipeptides, Z-L-Glu-L-Pro, shows enantioselectivity for the amino alcohols studied (metoprolol and analogues), although ion pairing occurred with both peptides. The possibility of using Z-L-Glu-L-Pro at temperatures above ambient has been investigated. In contrast to using L-ZGP, the enantioselectivity is, however, only slightly affected when increasing the temperature. Although the retention is decreasing at elevated temperatures, the enantiomers of, for example, metoprolol, may be separated within a wide temperature range, 10 to 45°C. An enantiomeric separation of metoprolol is given in **Figure 7**. It may also be worth noting that the elution order of the enantiomers were opposite using Z-L-Glu-L-Pro and L-ZGP as selectors, even though in both cases the terminal peptide was L-proline. The relative retention order of the studied amino alcohols was independent of the selector used, L-ZGP or Z-L-Glu-L-Pro.

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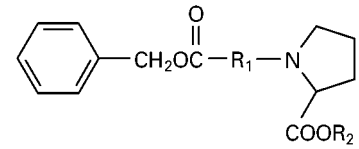
(−)-2,3:4,6-Di-O-isopropylidene-2-keto-L-gulonic Acid ((−)DIKGA)

(−)-DIKGA [**III**], has been used to resolve enantiomers by fractional crystallization and has also been

Table 4 Counterion structure and stereoselectivity



Counterion	R_1	R_2	k_R[b]	$\alpha_{S/R}$
Z-glycyl-L-proline (L-ZGP)	$HNCH_2CO$	H	4.4	1.24
Z-L-proline	–	H	3.2	1.15
Z-L-proline methylester[a]	–	CH_3	1.9	1.00
Z-L-alanyl-L-proline	$HNCH(CH_3)CO$	H	2.6	1.00
Z-L-prolyl-L-alanine			6.4	1.10

Solid phase: LiChrosorb DIOL. Mobile phase: 2.5 mM counterion and 1.0 mM triethylamine in dichloromethane (80 p.p.m. H_2O).
[a]Triethylamine 4.0×10^{-5} M in mobile phase. Reprinted from Pettersson C and Josefsson M (1986) *Chromatographia* 21: 321 with permission from the authors.
[b]Solute: *R*- and *S*-alprenol.

used as chiral selector in liquid chromatography. Several compounds including β-blocking agents, local anaesthetics and neuroleptics are resolved into their enantiomers when (−)-DIKGA is present in a polar mobile and PGC is used as support. An example is given in **Figure 8**. The enantiomers of *p*-hydro-

Table 5 Examples of different solutes separated in systems with L-ZGP as chiral counterion

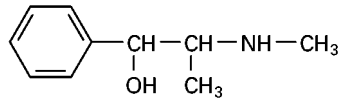
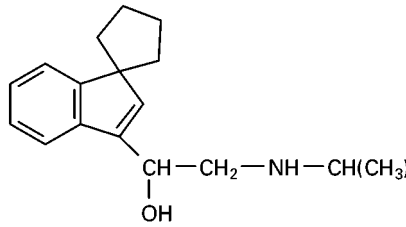
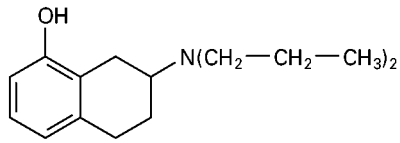
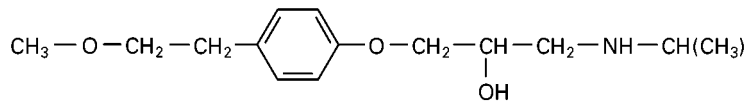
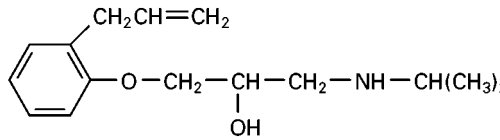
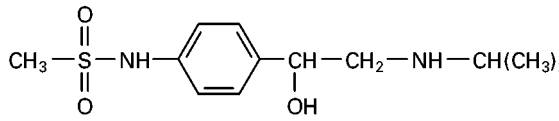
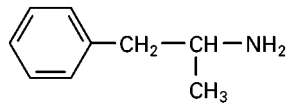
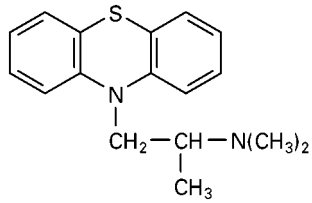
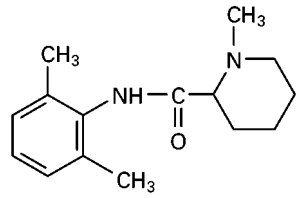
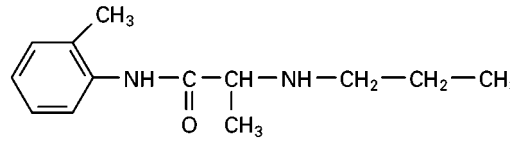
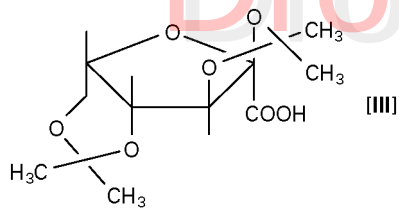
Solute	*Structure*	k_1	α
Ephedrine		7.06[a]	1.34
N-Isopropyl-2-hydroxy-2-(spirocyclopentane-1,1′-inden)-3′-ylethylamine		1.63[a]	1.57
8-Hydroxy-2-(di-n-propylamino) tetraline		2.24[a] (3.5[c])	1.12 (2.04[c])
Metoprolol		18[b] (2.4[c])	1.09 (1.25[c])
Alprenolol		22[b]	1.17
Sotalol		9.8[b]	1.13
1-Phenyl-2-aminopropane		3.8[c]	1.15
Promethazine		1.0[c]	1.15

Table 5 *Continued*

Solute	*Structure*	k_1	α
Mepivacaine		9.6[d]	1.35
Prilocaine		0.91[c]	1.12

[a]Solid phase: Nucleosil CN. Mobile phase: 2.5 mM L-ZGP and 0.25 mM triethylamine in dichloromethane (80 ppm water). Results from Pettersson C, Karlsson A and Gioeli C (1987) *Journal of Chromatography* 407: 217–229 with permission from Elsevier Science.
[b]Solid phase: PGC. Mobile phase: 5 mM L-ZGP and 4.5 mM NaOH in methanol. Results from Huynh NG, Karlsson A and Pettersson C (1995) *Journal of Chromatography* 705: 275–287 with permission from Elsevier Science.
[c]Solid phase: PGC. Mobile phase: 10 mM L-ZGP in dichloromethane (80 ppm water). Results from Karlsson A and Pettersson C (1991) *Journal of Chromatography* 543: 287–297 with permission from Elsevier Science and Karlsson A, Luthman K, Pettersson A and Hacksell U (1993) *Acta Chemica Scandinavica* 47: 469–481.
[d]Solid phase: PGC. Mobile phase: 5 mM L-ZGP in dichloromethane : hexane (1 : 1). Results from Karlsson A and Pettersson C (1992) *Chirality* 4: 323–332, reprinted by permission Wiley–Liss, Inc., a subsidiary of John Wiley and Sons, Inc.

xyephedrine are separated within 4 min.



[III]

General Mobile Phase Considerations

Beside the counterion and its concentration, the composition of the mobile phase will depend on the column packing material used and the type of solutes to analyse. Uncharged or charged modifiers may be needed in the mobile phase in order to elute the solutes in a reasonable time and also to resolve them into their enantiomers.

Mobile Phase Composition when using DIOL Functionalized Silica as Stationary Phase

When DIOL functionalized silica particles are used as stationary phase, the mobile phase has to be a non-polar solvent. The water concentration in the non-polar solvent may then be of vital importance for the success of the separation and should, together with some of the chiral counterions, quinine and camphorsulfonic acid, be as low as possible in order to promote the chiral separations. The equilibration time needed to obtain a stable system with dry solvent is, however, very long and therefore it is convenient to work with higher water contents although not a water-saturated mobile phase. The water content does not influence the enantioselectivity when L-ZGP is used as counterion. Longer retention times are, however, obtained when more water is present in the solvent. Water-saturated injection solutions can be injected into the system without influence on either retention or stereoselectivity.

As the polarity of the mobile phase controls the retention of the solutes, modifiers like alcohols, acetonitrile or tetrahydrofuran have sometimes to be used in order to elute solutes in a reasonable time. Several of these modifiers also have an effect on the enantioselectivities of these systems. Some modifiers have been studied in a system with (+)-10-camphorsulfonate in dichloromethane as mobile phase. (In these cases the mobile phases were prepared from dry dichloromethane.) It was found that the stereoselectivity was lower with hydrogen donating modifiers than with hydrogen accepting ones (**Table 7**). One hypothesis is that the modifiers may interact with the hydrogen-bonding groups in the selectors. It should be noted that 1-pentanol is preferred as a modifier since the peaks obtained are more symmetrical than

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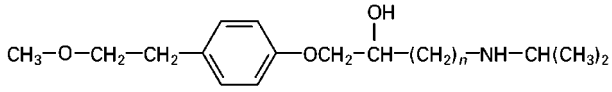
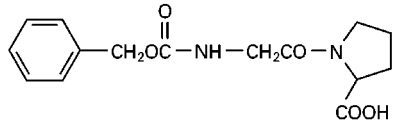
Table 6 Counterion structure and enantioselective retention

Solutes:

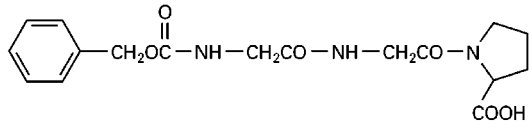


Counterions:

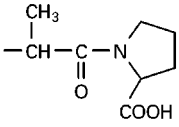
L-ZGP



L-ZGGP



Captopril



Solute (*n*)	*Counterion*					
	L-ZGP		*L-ZGGP*		*Captopril*	
	k_1	α	k_1	α	k_1	α
1	1.8	1.32	4.9	1.05	9.0	1.07
2	2.2	1.0	3.9	1.09	7.0	1.0
3	1.2	1.06	4.0	1.44	–	–

Solid phase: PGC. Mobile phase: 7.2 mM of counterion in dichloromethane (80 ppm H_2O). Reprinted from Karlsson A and Pettersson C (1992) *Chirality* 4: 323 with permission from Wiley-Liss, Inc., a subsidiary of John Wiley and Sons, Inc.

when using, for example, tetrahydrofuran. The improved peak shape is believed to be due to deactivation of strong adsorption sites on the silica surface.

Peak shapes may also be improved by adding to the mobile phase a compound with the same protolytic character as the solute enantiomers. Thus, for the separation of amines, triethylamine is often used to improve peak symmetry. Acidic compounds to be analysed with, for example, quinine, may need addition of acid to the mobile phase in order to effect elution. The added acid is believed to compete for adsorption to the solid phase and for ion pair formation with quinine.

Mobile Phase Composition when using PGC as Stationary Phase

When PGC is used as stationary phase, both nonpolar and polar mobile phases may be used. The nonpolar

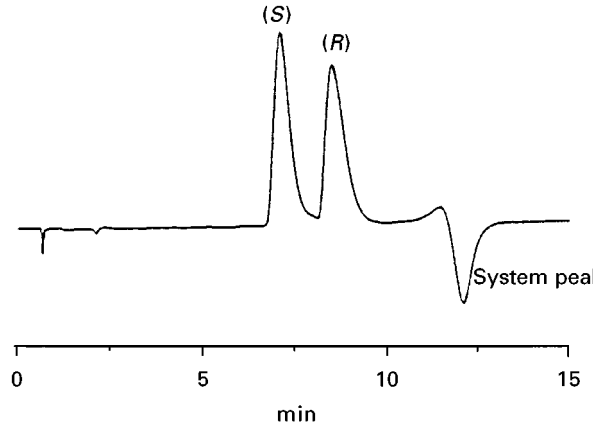


Figure 7 Separation of (*R*)- and (*S*)-metoprolol. Solid phase: Hypercarb. Mobile phase: 3.4 mM Z-L-Glu-L-Pro and 5.5 mM NaOH in methanol. Column temperature: 35°C. (Reprinted from Karlsson A and Karlsson O (1997) Enantiomeric separation of amino alcohols using Z-L-Glu-L-Pro or Z-L-Glu-D-Pro as chiral counterions and Hypercarb as the solid phase. *Chirality* 9: 650–655, with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

phases resemble those used with the DIOL stationary phase. The addition of a nonchiral hydrophobic acid may, however, be needed in order to elute some of the hydrophobic acids from the PGC. Naproxen was found to be too strongly retained using a mobile phase consisting of quinine in dichloromethane and hexane. When adding β-naphthalenecarboxylic acid to the mobile phase, naproxen was eluted within a reasonable time and the enantiomers were separated (**Figure 9**). The effect of addition of an acid to the mobile phase is not easy to predict as the added acid may compete for both the distribution to limited adsorption sites on the support and also for ion pair formation with the chiral selector. Furthermore, if the counterion added to the polar mobile phase is expected to remain in an uncharged form, an acid or base has to be added in order to obtain a charged counterion. L-ZGP has a pK_a of 7–9 in methanol. The amount of base added (e.g., sodium hydroxide) will determine the degree of protolysis of the L-ZGP. However, an excess of base will result in an alkaline solution where the solutes may be uncharged. The added base may thereby influence the retention and the stereoselectivity, in other words influence both k and the ratio $k_{achiral}/k_{chiral}$ (Figure 3).

Conclusions

The addition of a counterion to the mobile phase is a technique often used to control the retention of solutes. If the added counterion is chiral, chiral separations may be achieved provided that appropriate

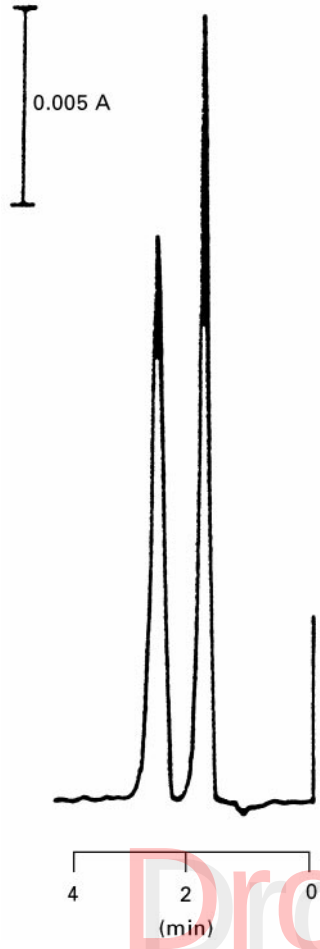


Figure 8 Separation of rac-*p*-hydroxyephedrine. Solid phase: Hypercarb. Mobile phase: 20 mM (−)-DIKGA and 15 mM NaOH in isopropanol : acetonitrile (9 : 1). Flow rate: 1.0 mL min^{-1}. (Reprinted from Pettersson C and Gioeli C (1993) Chiral separation of amines using reversed-phased ion-pair chromatography. *Chirality* 5: 241–245, with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

solvents and solid supports are chosen. Using mobile phase additives is an easy way to screen for new chiral selectors. Furthermore, if the chiral counterion is

Table 7 Influence of polar components in the mobile phase

Polar solvent	*Content (%)*	k_1	$\alpha_{+/-}$	*Asf*[a]
Pentanol	0.5	20.3	1.08	1.5
	1	10.8	1.06	1.1
	5	2.02	1.02	1.1
Isopropanol	0.5	13.5	1.07	2.6
Acetonitrile	1	25.1	1.09	2.2
Tetrahydrofuran	1	11.9	1.10	3.7
Ethyl acetate	1	29.0	1.10	3.6

Sample: Alprenolol (+ and − forms). Mobile phase: (+)-10-camphorsulfonate, 2.1 mM in dichloromethane–polar solvent. [a]Asf = asymmetry factor measured at baseline. Back part of peak/front part of peak. Results from Pettersson C and Schill G (1981) *Journal of Chromatography* 204: 179–183, with permission from Elsevier Science.

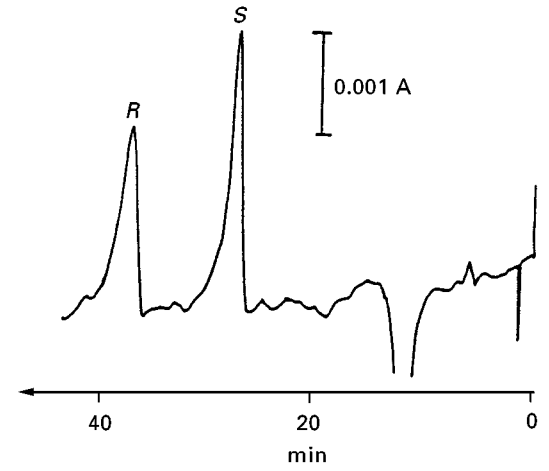


Figure 9 Resolution of (*R*,*S*)-naproxene. Solid phase: PGC. Mobile phase: 0.35 mM quinine and 0.10 mM β-dinaphthalenecarbonic acid in dichloromethane : hexane (1 : 1) (Reprinted from Karlsson A and Pettersson C (1992) Separation of enantiomeric amines and acids using chiral ion-pair chromatography on porous graphitic carbon. *Chirality* 5: 323–332, with permission from Wiley-Liss, Inc., a subsidiary of John Wiley and Sons, Inc.)

available in both its enatiomeric forms, the retention order of a pair of enantiomers may be controlled. In contrast to derivatization with chiral reagents, there is no need for optically pure counterions or counterions with exactly known purities. An optically impure selector will, in chiral ion pair chromatography, still give enantiomerically pure peaks, although eluting closer to each other than they would if an optically pure selector were used.

See also: **II/Chromatography: Liquid:** Ion Pair Liquid Chromatography. **Chromatography: Thin-Layer (Planar):** Ion Pair Thin-Layer (Planar) Chromatography. **III/Chiral Separations:** Capillary Electrophoresis; Chiral Derivatization; Cyclodextrins and Other Inclusion Complexation Approaches; Ligand Exchange Chromtography; Liquid Chromatography; Molecular Imprints as Stationary Phases; Protein Stationary Phases; Synthetic Multiple Interaction ('Pinkle') Stationary Phases.

Further Reading

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Josefsson M, Carlsson B and Norlander B (1994) Chiral ion-pair chromatographic separation of two dihydropyridines with camphorsulfonic acids on porous graphitic carbon. *Journal of Chromatography* 684: 23–27.

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Ligand Exchange Chromatography

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Introduction

Ligand exchange chromatography (LEC) was first introduced by Helfferich in 1961 as a general chromatographic technique to separate compounds which are able to form labile complexes with transition metal cations. The basic idea was to immobilize such ions as Cu(II) or Ni(II) on a stationary phase, in particular, a cation exchanger with sulfonic or carboxylic functional groups, and then let the ions form labile coordination compounds with analytes which possess electron-donating functional groups and can enter the coordination sphere of the metal ion, thus acting as ligands. Those analytes that form stronger complexes with the central ion, are retained longer on such a metal ion-incorporating stationary phase.

Starting with the idea that, in the densely packed coordination sphere, ligands enter multipoint interactions with each other and should therefore mutually recognize the spatial configuration of neighbours, Davankov and Rogozhin (1968–71) synthesized chiral complex-forming resins and further developed LEC into a powerful chiral chromatographic method. This technique, for the first time in liquid chromatography, resulted in a complete and reliable resolution of a racemate into constituent enantiomers. Typical analytes for enantioseletive LEC are members of such important classes of chiral compounds as amino acids, hydroxy acids and amino alcohols. Having soon become one of the most extensively investigated methods for the direct resolution of enantiomers, LEC maintained for a long period of time its leading positions in developing novel chiral chromatographic systems and evaluating mechanisms of chiral recognition and discrimination. This technique contributed much to the successful development of chiral silica-bonded stationary phases, both monomeric and polymeric, chiral coatings on column-packing materials and chiral mobile phase additives. In this last technique, it transpired that the central metal ion does not need to reside in the stationary phase, but, instead, can be a part of a chiral complex that is doped into the mobile phase to interact there with the analytes. This widens significantly the definition of LEC to a chromatographic process in which the formation and breaking of labile coordinate bonds to a central metal cation are responsible for the separation of complex forming analytes.

Ligand exchange has been realized in all known modes of enantioselective chromatography, including liquid chromatography, thin layer chromatography, gas chromatography, capillary electrophoresis and countercurrent liquid chromatography. In gas chromatography the principle of ligand exchange is more commonly known under the name 'complexation chromatography'.

Theoretical Aspects of Chiral Discrimination in LEC

Being built of protons, neutrons and electrons, which basically are chiral elementary species, all atoms possess inherent chirality. Therefore, two enantiomeric molecules resulting from a mirror-symmetric arrangement of a certain ensemble of atoms in space, can differ in their thermodynamic stability. However, this difference may only amount to about 10^{-14} J mol^{-1}, which practically makes the enantiomers energetically identical and indistinguishable in any achiral environment. Using any chromatographic technique, two enantiomers can be recognized and